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(54) Title: ASSAY METHOD

(57) Abstract

A method of assaying for an analyte by the use of surface plasmon resonance spectrometry (SPRS). The analyte is a member of a non-immune ligand-receptor pair. A metal surface carries the analyte or an analogue immobilised with the other member of the ligand-receptor pair reversibly bound thereto. A fluid containing the analyte is brought into contact with the metal surface, and displacement of the other member of the ligand-receptor pair monitored by SPRS.

* See back of page

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ASSAY METHOD

This invention concerns a method of assaying for a macromolecular analyte by use of the technique of surface plasmon resonance spectrometry (SPRS).

The phenomenon of SPR is well known and will not be described in detail (see EPA 305109 for example). Briefly, the intensity of monochromatic plain-polarised light (conveniently obtained from a laser) reflected from the interface between an optically transparent material, e.g. glass, and a thin metal layer depends on the refractive index of material on the downstream side of the metal. Accordingly, by measuring changes in intensity of the reflected light an indication can be obtained of changes in refractive index of material at a particular point on the down-stream surface of the metal. The intensity of reflected light also varies with the angle of incidence, and reflectivity drops sharply to a minimum at a particular angle which is characteristic of the equipment.

WO 89/08260 describes a method of analysing for an analyte in a sample by bringing the sample into contact with a metal surface, on which an antibody has previously been reversibly bound to immobilised analyte or analogue, and monitoring displacement of antibody as indicative of the presence or the concentration of the analyte in the sample. That specification is mainly concerned with hapten analytes and describes only hapten-antibody and antigen-antibody binding pairs.

EPA 276142 describes competition assays involving an analyte and two other reagents, in which SPRS is used to monitor the formation of a complex on a solid surface. The assay involves the use of at

least one other liquid reagent in addition to the sample. The signal resulting from complex formation on the surface may be obscured by noise resulting from non-specific binding of other macromolecules to the surface.

This invention provides a method of assaying for an analyte, preferably a macromolecular analyte, which is a member of a ligand-receptor pair other than a hapten-antibody or an antigen-antibody pair, by the use of a metal surface adapted for surface plasmon resonance spectrometry which metal surface carries the analyte or an analogue thereof immobilised thereon with the other member of the ligand-receptor pair reversibly bound thereto, which method comprises bringing a fluid containing the analyte into contact with the metal surface and observing by surface plasmon resonance spectrometry displacement of the other member of the ligand-receptor pair from the surface. The analyte is a member of a ligand-receptor pair. Many examples of such pairs are known and include the following:

Ligand	Receptor
DNA	DNA
DNA	RNA
RNA	RNA
Protein	DNA
Protein	RNA
DNA	Binding drug
RNA	Binding drug
Lectin	Oligosaccharide (free or in a glycoprotein or glycolipid)
Neurotransmitter or analogue	Protein receptor
Hormone	Hormone receptor
Growth factor or analogue	Protein receptor

Differentiation factor or analogue	Protein receptor
Enzyme	Cofactor, substrate or inhibitor
Biotin	Avidin
Enzyme	Prosthetic group
Oligopeptide	Protein
Immunoglobulin	Protein A
Drug	Binding protein

In the above, the term protein is used to include peptides.

The method involves the use of a metal surface adapted for surface plasmon resonance spectrometry. The metal may comprise silver or gold, conveniently in the form of a layer e.g. deposited by evaporation on a carrier such as a glass slide. The metal surface carries the analyte or an analogue thereof immobilised thereon. An analogue of the analyte is a substance which competes with the analyte for binding to the other member of the ligand-receptor pair. Often the analogue will be arranged to be as near as possible or even completely identical to the analyte. The use in assays of analyte analogues is well known. Binding of the analyte or analogue to the metal surface without loss of binding power is effected by methods which are well known. Particularly when the analyte or analogue is of low molecular weight, the use of a spacer molecule may be required. To prevent non-specific binding at a later stage in the assay, any surplus area of the metal surface may be coated e.g. with an inert protein.

Reversibly bound to the immobilised analyte or analogue is the other member of the ligand-receptor pair. In another aspect, this invention provides an assay device comprising a metal surface adapted for surface plasmon resonance spectrometry, which surface

carries immobilised thereon a member of a ligand-receptor pair, other than a hapten-antibody or an antigen-antibody pair, with the other member of the ligand-receptor pair reversibly bound thereto. That other member of the ligand-receptor pair may have been modified to increase the signal generated to increase the SPR signal generated by its removal from the metal surface. Such modification may involve addition of a molecule or group of low refractive index, or more preferably high refractive index such as polystyrene, titanium dioxide or gold colloid.

Using this pre-formed device, the method of the invention is very simple. A fluid containing the analyte is brought into contact with the pre-coated metal surface. Analyte in the fluid sample competes with immobilised analyte for binding to the other member of the ligand-receptor pair. Displacement of the other member of the ligand-receptor pair into solution, as a complex with analyte in the sample, is monitored by surface plasmon resonance spectrometry. The method has two particular advantages:

(a) The only fluid reagent involved is the sample containing the analyte. When this is brought into contact with the coated metal surface, the presence or the concentration of the analyte in the sample can be assayed within minutes or even seconds.

- the SPRS signal, generated by removal of a reagent from the metal surface, is not significantly contaminated by noise due to non-specific binding of material to the surface.

Example 1

Displacement Assay using Nucleic Acid Probes

An oligonucleotide A (97mer) was hybridised to a complementary oligonucleotide B (17mer). Oligonucleotide B was then covalently linked to the silver surface of a slide. The slide was then blocked with hybridisation buffer. A further oligonucleotide C (50mer) which had the same sequence as part of oligonucleotide B and therefore complementary to A, was then added and displacement from the silver slide of the hybridised oligonucleotide A was measured. Results using ^{32}P end-labelled oligonucleotide A showed that as the amount of oligonucleotide C was increased more oligonucleotide A was released. Such changes will also be measurable by SPR.

Example 2

DNA Strand Displacement by SPR

Method

Preparation of oligonucleotides

A sixteen mer probe and a complementary ninety-seven mer target were prepared using phosphoramidite chemistry on the Applied Biosystems Model 380D DNA synthesizer. The DNA probe was modified in order to permit efficient and stable binding to the lver surface. This was prepared by attaching a terminal primary amine at the 5´-end of the molecule. The amino-oligonucleotide was mixed with an equal volume of 0.1M sodium hydrogen carbonate solution pH 8.5 and to this mixture a solution of SPDP (0.25 mg for each 1.0 OD of oligo used) in DMF was added. The reaction was left to equilibriate for 90 minutes at

room temperature and then eluted through a Sephadex G25 PD10 column. Fractions containing the required product were pooled together and purified by preparative HPLC. After purification the appropriate fractions were dried down by vacuum centrifugation to remove HPLC solvents and then reconstituted in a known volume of water.

Preparation of DNA Hybrid

DNA hybrid consisting of the modified 16 mer and a complementary 97 mer was prepared by mixing 16 mer and 97 mer in a 1.5:1 molar ratio ensuring that the 16 mer, the immobilisable probe, was in excess. The preannealing was carried out at 65°C in 2x SSPE (300mM NaCl, 20mM $NaH_2PO_4.H_2O$, 2mM ethylenediaminetetra-acetic acid) and the mixture was allowed to cool down to room temperature for 2hrs.

SPR Experiment

Using 1.8×10^{-10} moles of hybrid/ml, 1ml was pumped across a silver slide at 1µl/s after priming the slide with 2x SSPE. Following a wash step with 1ml of 2x SSPE the slide was blocked with hybridisation buffer followed by a 2x SSPE wash. 3×10^{-10} moles of a 50 mer, which is complementary in sequence to the 97 mer, in 1ml 2x SSPE was flowed across the slide at a speed of 1µl/s, and the change in reflectivity was monitored with time.

Control reactions involving no DNA and non-complementary DNA were carried out.

Data is presented below with the No DNA control value deducted from the experimental.

Table 1

Target DNA	% change in reflectivity
No DNA control	0
50 mer	-1
Non-complementary DNA	+1

Results demonstrate displacement of the 97 mer sequence away from the silver surface by the complementary 50 base sequence. This does not occur in the absence of this oligonucleotide nor in the presence of the non-complementary sequence which does not hybridise to the 97 mer.

CLAIMS

1. A method of assaying for an analyte which is a member of a ligand-receptor pair other than a hapten-antibody or an antigen-antibody pair, by the use of a metal surface adapted for surface plasmon resonance spectrometry which metal surface carries the analyte or an analogue thereof immobilised thereon with the other member of the ligand-receptor pair reversibly bound thereto, which method comprises bringing a fluid containing the analyte into contact with the metal surface and observing by surface plasmon resonance spectrometry displacement of the other member of the ligand-receptor pair from the surface.

2. A method as claimed in claim 1, wherein the analyte is a macromolecule.

3. An assay device comprising a metal surface adapted for surface plasmon resonance spectrometry, which surface carries immobilised thereon a member of a ligand-receptor pair, other than a hapten-antibody or an antigen-antibody pair, with the other member of the ligand-receptor pair reversibly bound thereto.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 90/01319

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: G 01 N 33/58, 21/55

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	G 01 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X.	EP, A0, 276142 (ARES-SERONO RESEARCH & DEVELOPMENT LIMITED PARTNERSHIP) 27 July 1988, see page 1, line 1; page 3, line 20 table 1; cited in the application	1-3
X	WO, A, 8703093 (RADIOMETER A/S) 21 May 1987, see page 6, line 34; page 7, line 20; page 9, line 1 - line 18; claims 1,7-9	1-3
P,X	WO, A, 8909408 (ARES-SERONO RESEARCH & DEVELOPMENT LIMITED PARTNERSHIP) 5 October 1989, see page 5, line 18; page 6, line 1; page 8, line 8 - line 20 table 2, claims	1-3

* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12th November 1990	11. 12. 90
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	miss T. MORTENSEN

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	US, A, 4626513 (J A BURTON ET AL) 2 December 1986, see column 1, line 5 - line 48; column 4, line 22 - line 26; column 8, line 54; column 9, line 45 claims	1-3
Y	EP, A, 0245206 (BATTELLE MEMORIAL INST) 11 November 1987, see page 21, line 1 - line 8; page 24; claims 1,28-29	1-3
P,Y	WO, A, 8908260 (AMERSHAM INT PLC) 8 September 1989, see page 4, line 5; page 6, line 20 claims; cited in the application	1-3

AN TO THE INTERNATIONAL SEARCH REPORT
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SA 39697

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent ffice EDP file on 27/09/90
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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A0- 276142	27/07/88	NONE		
WO-A- 8703093	21/05/87	EP-A-	0245396	19/11/87
WO-A- 8909408	05/10/89	AU-D-	3361889	16/10/89
		EP-A-	0359807	28/03/90
US-A- 4626513	02/12/86	WO-A-	88/04429	16/06/88
EP-A- 0245206	11/11/87	AU-D-	7583887	01/12/87
		JP-T-	1500221	26/01/89
		WO-A-	87/06956	19/11/87
WO-A- 8908260	08/09/89	AU-D-	3077489	31/08/89
		EP-A-	0378594	25/07/90

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